Body Size, Physical Activity, Early-Life Energy Restriction, and Associations with Methylated Insulin-like Growth Factor-Binding Protein Genes in Colorectal Cancer

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Research Article

Body Size, Physical Activity, Early-Life Energy Restriction, and Associations with Methylated Insulin-like Growth Factor–Binding Protein Genes in Colorectal Cancer

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Abstract

Background: We investigated body size, physical activity, and early-life energy restriction in relation to colorectal tumors with and without methylated insulin-like growth factor–binding protein (*IGFBP*) genes, which are putative tumor-suppressor genes.

Methods: We determined *IGFBP2*, *IGFBP3*, and *IGFBP7* promoter CpG island hypermethylation in tumors of 733 colorectal cancer cases from the Netherlands Cohort Study ($N = 120{,}852$). Participants self-reported lifestyle and dietary factors at baseline in 1986. Using a case–cohort approach (N subcohort = 5,000), we estimated hazard ratios (HR) for colorectal cancer by extent of *IGFBP* methylation.

Results: Comparison of the highest versus lowest sex-specific tertiles of adult body mass index (BMI) gave multivariable-adjusted HRs [95% confidence intervals (CI)] for colorectal cancers with 0 (18.7%), 1 (29.5%), 2 (32.4%), and 3 (19.5%) methylated genes of 1.39 (0.88–2.19), 1.11 (0.77–1.62), 1.67 (1.17–2.38), and 2.07 (1.29–3.33), respectively. Other anthropometric measures and physical activity were not associated with colorectal cancer risk by extent of *IGFBP* methylation, except height in sex-specific analyses for women. Exposure to energy restriction during the Dutch Hunger Winter versus nonexposure gave HRs (95% CIs) for colorectal cancers with 0, 1, 2, and 3 methylated genes of 1.01 (0.67–1.53), 1.03 (0.74–1.44), 0.72 (0.52–0.99), and 0.50 (0.32–0.78), respectively.

Conclusions: Adult BMI, height (in women only), and early-life energy restriction were associated with the risk of having a colorectal tumor characterized by *IGFBP* methylation.

Impact: Body size may particularly increase the risk of *IGFBP* gene–methylated colorectal tumors; this finding might facilitate more targeted approaches to prevent obesity-related colorectal cancers. *Cancer Epidemiol Biomarkers Prev; 23(9); 1852–62. ©2014 AACR.*

Introduction

The insulin-like growth factor (IGF) pathway regulates growth by affecting cell proliferation, differentiation, and apoptosis, and has long been thought one of the mechanisms through which overweight and a lack of physical activity increase colorectal cancer risk (1). The IGF pathway includes IGFI and -II, of which IGFI is the main growth factor in adult life, IGF binding proteins (IGFBP-1–6), and IGFBP-related proteins (IGFBP-7–10).

We focus on IGFBP-1–7, which have been most studied. In the context of this article, we view IGFBPs as putative tumor suppressors with local effects in colorectal tissue. Traditionally, IGFBPs have been viewed as carrier proteins that transport IGFs through the circulatory system, extending their half-lives and preventing them from reaching target tissues until released. In this way, IGFBPs regulate IGF bioavailability (2, 3). Today, IGFBPs have been shown to have numerous actions that can be endocrine (via the circulatory system), paracrine, autocrine, and intranuclear. Actions can be IGF-dependent and IGF-independent (i.e., IGFBPs may act alone or bind to other molecules; refs. 4, 5). Growth stimulatory effects are not excluded, although IGFBPs are best known for their growth-suppressing effects (4,5).

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Considering their role as tumor suppressors, silencing of *IGFBP* genes through promoter hypermethylation likely counteracts the growth inhibitory effect conferred by IGFBPs. The evidence for this includes studies showing growth inhibition in cell lines of hepatocellular, breast, and melanoma cancer characterized by promoter hypermethylation of *IGFBP3* in response to demethylating agents and/or histone deacetylase inhibitors (5). *IGFBP* methylation has, furthermore, been indicated an unfavorable event in cancer development, including colorectal cancer development, by cell studies investigating methylation of *IGFBP1* (6), *IGFBP2* (7), *IGFBP3* (6), *IGFBP4* (7), and *IGFBP7* (8–11). Finally, *IGFBP1* methylation levels in peripheral blood were recently found increased in type II diabetic men compared with normal glucose-tolerant individuals (12). Type II diabetics as compared with nondiabetics have been found to be at an increased risk of colorectal cancer (13).

Because methylation is an early event in colorectal tumorigenesis (14), we hypothesized that promoter CpG island hypermethylation of *IGFBP* genes could sensitize individuals to the effects of colorectal cancer risk factors such as overweight and physical activity. Therefore, within the prospective Netherlands Cohort Study (NLCS), we studied body size, physical activity, and early-life energy restriction in relation to the extent of *IGFBP* methylation in colorectal tumors. We hypothesized that a larger body size increases the risk of having a colorectal tumor with methylated *IGFBP* genes but not without methylated *IGFBP* genes. We hypothesized that physical activity and early-life energy restriction decrease the risk of having a colorectal tumor with methylated *IGFBP* genes but not without methylated *IGFBP* genes. In addition, we explored relationships between *IGFBP* methylation, microsatellite instability (MSI; ref. 15), and the CpG island methylator phenotype (CIMP; ref. 16), because MSI and CIMP involve methylation of several tumor suppressor- and DNA repair genes. *IGFBP* methylation might, therefore, tag MSI or CIMP phenotypes, which may be reflected in correlations between *IGFBP* methylation on the one hand and MSI and CIMP phenotypes on the other hand.

Materials and Methods

Study population and design

The NLCS (17) includes 120,852 participants, sampled from 204 Dutch municipalities, who were 55 to 69 years old at baseline in 1986. At baseline, participants completed a self-administered questionnaire, including a semi-quantitative 150-item food frequency questionnaire (FFQ). The FFQ was found to rank individuals adequately according to dietary intake as compared with a 9-day dietary record (18) and was shown a good indicator of intake for at least 5 years (19,20). Participants who reported a history of cancer (other than skin cancer) were excluded. The NLCS was approved by the review boards of the TNO Nutrition and Food Research Institute and Maastricht University in the Netherlands.

The NLCS is characterized by a case–cohort approach. This approach entails that a random subcohort ($N = 5{,}000$)—selected immediately after baseline and representative of the whole cohort—is followed up to estimate the person-time at risk, whereas incident cancer cases are enumerated for the entire cohort. Subcohort members contribute to the person-time at risk until the end of follow-up, cancer incidence, death, or loss to follow-up. Follow-up for vital status is performed through linkage to the Central Bureau of Genealogy and the municipal population registries (~100% completeness). Cancer follow-up is performed through linkage with the population-based cancer registry and PALGA (Netherlands pathology database; >96% completeness; refs. 21–23). In the period 1989 to 1993 (the follow-up period used for the current analyses), 939 incident colorectal cancer cases occurred. Sufficient tumor DNA, isolated from formalin-fixed, paraffin-embedded sections after macro-dissection of tumor cells, was available for 733 cases (Fig. 1). Age at diagnosis, tumor sublocalization (ICD-O-1 153), and tumor node metastasis (TNM) stage were retrieved from the cancer registry.

Body size, physical activity, and early-life energy restriction

Adult body mass index [BMI; weight (kg)/height $(m)^2$], BMI at age 20, BMI change since age 20, height (cm), and adult trouser/skirt size were derived from the baseline questionnaire. Adult BMI, BMI at age 20, and height were categorized into sex-specific tertiles. We deviated from the BMI categorization of the World Health Organization (WHO) that distinguishes between underweight (<18.5 kg/m^2), normal weight (18.5–<25 kg/m^2), overweight (25–<30 kg/m^2), and obese individuals ($\geq$30 kg/m^2; ref. 24), because of power considerations. Adult trouser/skirt size was shown to correlate well with hip and waist circumferences in a subset of weight-stable individuals, and was associated with endometrial and renal cell cancer risk in a fashion as would be expected for waist circumference, rendering it a good proxy (25).

Nonoccupational physical activity at baseline in minutes per day was a sum measure of several activities. It included daily walking/cycling (min/d), weekly recreational walking/cycling, weekly engagement in gardening/odd jobs, and weekly participation in sports/gymnastics (categories: never, 1, 1–2, and >2 h/wk). Categories were $\leq$30, >30–60, >60–90, and >90 min/d. In the present article, the two middle categories were combined for power considerations. Occupational physical activity was derived from self-reported occupational history on the basis of a rating system developed by Hettinger (26), which distinguishes between jobs with an energy expenditure of <8, 8–12, and >12 kJ/min. Occupational physical activity was representative of long-term physical activity in men and used in sex-specific analyses for men only, because a substantial number of women were never employed or only in the distant past (27).

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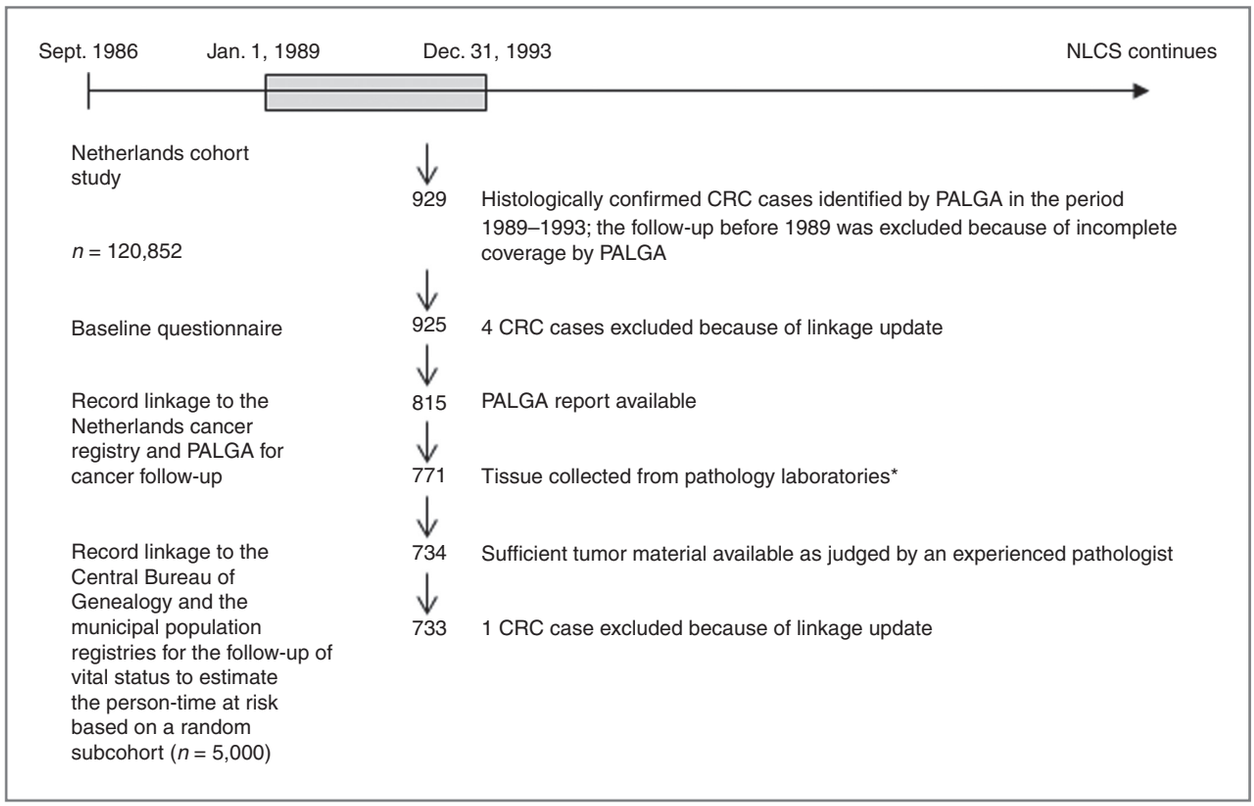

Figure 1. Flow chart of colorectal cancer cases available for analysis. CRC, colorectal cancer. *, Tumor tissue was collected after approval by the ethical review boards of Maastricht University, the population-based cancer registry, and PALGA; the pathology laboratories made available the tumor blocks between August 1999 and December 2001.

Early-life energy restriction was measured through three proxy variables: the place of residence during the Hunger Winter (1944–45); the place of residence in 1942, reflecting the war years (1940–44); and the employment status of an individual's father during the economic depression (1932–40). Nutritional differences during these periods have been well documented in the Netherlands. In particular, living in a Western city in the Netherlands during the Hunger Winter indicated severe energy restriction. The Dutch Hunger Winter was preceded by a German food embargo and was unusually early and harsh; by the time the embargo was (partially) lifted, the canals had frozen over, which made it impossible to transport food into the Western parts of the country, and 11 cities are considered famine cities: Amsterdam, Rotterdam, The Hague, Utrecht, Zaandam, Hilversum, Amersfoort, Dordrecht, Vlaardingen/Schiedam, Delft, and Leiden. At the height of the famine, from December 1944 to April 1945, official daily rations per capita were between 400 and 800 kilocalories, although the diet remained nutritionally balanced (28, 29). During the economic depression, sufficient calories were available, but the variation in the food pattern had likely been limited if an individual's father was unemployed. NLCS participants were between 12 and 28 years old during the Hunger Winter, between 8 and 28 years old during the war years, and between 0 and 23 years old during the economic depression.

Data on adult BMI, BMI at age 20, BMI change, adult trouser/skirt size, height, nonoccupational physical activity, occupational physical activity, place of residence during the Hunger Winter, place of residence during World War II, and employment status of an individual's father during the economic depression were complete for 96.1%, 82.0%, 81.8%, 93.4%, 96.7%, 98.0%, 87.1%, 87.3%, 72.6%, and 93.9% of subcohort members, respectively.

Laboratory analyses

IGFBP methylation. We determined *IGFBP2*, *IGFBP3*, *IGFBP4*, and *IGFBP7* CpG island promoter hypermethylation by methylation-specific PCR (MSP; refs. 30, 31) after bisulfite modification of 500 ng DNA (Zymo Research), because methylation had been reported in these *IGFBP* genes before this study. To facilitate MSP on DNA retrieved from formalin-fixed, paraffin-embedded tissue, DNA was first amplified with flanking PCR primers that amplify bisulfite-modified DNA, but do not preferentially amplify methylated or unmethylated DNA. The resulting fragment was used as a template for the MSP reaction. All PCRs were carried out with controls for unmethylated alleles (DNA from normal lymphocytes), methylated alleles [normal lymphocyte DNA treated *in*

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vitro with SssI methyltransferase (New England Biolabs)], and a control without DNA. Ten microliters of each MSP reaction was directly loaded on to a nondenaturing 2% agarose gel, stained with gelstar, and visualized under UV illumination. Primers were designed on the basis of a deep sequencing analysis of methylation in colon cancer cell lines, to precisely cover densely methylated CpG islands (14). *IGFBP4* methylation analyses were discontinued after preliminary results showed methylation in only four out of 100 samples. We concluded that *IGFBP4* methylation is rare in colorectal cancer and that analyses would not be cost effective. The primer sequences used to analyze *IGFBP2*, *IGFBP3*, and *IGFBP7* are shown in Supplementary Table S1. Methylation analyses of *IGFBP2*, *IGFBP3*, and *IGFBP7* were successful in 98.9%, 94.4%, and 98.0% of 733 colorectal cancer cases, respectively; reproducibility in duplo or triplo analyses was 93.2% ($N = 103$ sample pairs/trios), 95.1% ($N = 122$ sample pairs/trios), and 84.4% ($N = 77$ sample pairs/trios), respectively.

CIMP and MSI. CIMP was defined by CpG island promoter hypermethylation of ≥3 out of five Weisenberger markers (*CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3*, and *SOCS1*; ref. 32). Methylation of these markers was analyzed using MSP as described previously (33). Analyses were successful in 81%, 79%, 79%, 90%, and 83% of 733 cases for *CACNA1G*, *IGF2*, *NEUROG1*, *RUNX*, and *SOCS1*, respectively. MSI was determined by a common approach: a pentaplex PCR using the mononucleotide repeats BAT-26, BAT-25, NR-21, NR-22, and NR-24. Allelic size variations in ≥3 repeats were a marker for MSI; other tumors were classified as microsatellite stable (MSS; ref. 34). Analyses were successful in 90% of 733 cases.

Statistical analysis

We used Cox regression to estimate hazard ratios (HR) and 95% confidence intervals (CI) for colorectal cancer risk by extent of *IGFBP* methylation in relation to body size, physical activity, and early-life energy restriction using Stata (Stata Corp.). The risk of colorectal cancer by methylation status of *IGFBP2*, *IGFBP3*, and *IGFBP7* was estimated for future literature comparisons. To account for the additional variance introduced by sampling the subcohort from the entire cohort, standard errors were estimated using the robust Huber–White sandwich estimator. A P value < 0.05 for two-sided testing indicated statistical significance. The proportional hazards assumption was tested using the scaled Schoenfeld residuals and by visually inspecting the -log-log-transformed hazard curves. No violations were detected.

We modeled risk with an age- and sex-adjusted model, and with multivariable-adjusted models, which included predefined potential confounders. Other potential confounders were included if these changed HRs by >10%. None of the variables considered did [family history of colorectal cancer, smoking status, socioeconomic status, diabetes, total energy intake, and intake of alcohol, meat, processed meat, fruit, vegetables, fiber, fat (energy-adjusted), water through foods and fluids, supplements, folate, beta-carotene, vitamin B6, vitamin C, vitamin E, calcium, iron, magnesium, flavonoids, and catechins]. The adjustments made in each model are presented in Table 1. With respect to BMI change, analyses were stratified by adult BMI (<25 and ≥25 kg/m^2) in an effort to disentangle the potential effect of weight gain on colorectal cancer risk from that of being overweight or obese in adulthood (BMI change correlated with adult BMI in subcohort members, Pearson $r = 0.68$; $P < 0.001$). Individuals with a negative BMI change were excluded in this analysis ($N = 492$), as there were too few cases among these individuals ($N = 51$ in total) to establish a separate category within strata of adult BMI and by extent of *IGFBP* methylation. Separating this group may be important as some weight gain with age may be expected, and severe weight loss might even indicate preclinical disease (the mean BMI change in subcohort members was 3.5 kg/m^2 with a SD of 3.4 kg/m^2).

Sensitivity analyses were performed to check for independent effects of adult BMI and BMI at age 20, a mediating effect of BMI at age 20 in models for early-life energy restriction, and a potential influence of timing of exposure to early-life energy restriction. An overview is given in Table 1. We also performed sex-specific analyses, even though the power to detect associations was limited, because previous NLCS data about colorectal cancer risk showed heterogeneous results for men and women (27, 35, 36).

Results

Population characteristics

Methylation of *IGFBP* genes was successfully analyzed in 652 colorectal cancer cases. The prevalence of *IGFBP2*, *IGFBP3*, and *IGFBP7* methylation was 40.6% ($N = 265$), 40.2% ($N = 262$), and 71.8% ($N = 468$), respectively. An *IGFBP* methylation index showed that 18.7% ($N = 122$) of colorectal cancer cases had 0 methylated genes, 29.5% ($N = 192$) had 1 methylated gene, 32.4% ($N = 211$) had 2 methylated genes, and 19.5% ($N = 127$) had 3 methylated genes. The investigated exposures did not evidently differ between subcohort members and colorectal cancer cases (Table 2).

The distribution of colorectal cancer cases across the *IGFBP* methylation index significantly differed from that across instability types as based on CIMP and MSI status ($P < 0.001$; Table 3). The percentage of MSI tumors (of which 76.9% also had CIMP) and MSS CIMP tumors increased with an increasing number of methylated *IGFBP* genes (+17.4% and +36.3%, respectively), whereas the percentage of MSS non-CIMP tumors decreased across *IGFBP* index groups (−53.7%). All three instability groups were present among colorectal cancers with 3 methylated *IGFBP* genes: 24.5% of colorectal cancer cases were MSI tumors, 38.7% were MSS CIMP tumors, and 36.8% were MSS non-CIMP tumors. With increasing methylated *IGFBP* gene numbers, the tumor location was more often proximal and less often distal ($P < 0.001$). Age at diagnosis and TNM stage did

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Table 1. Overview of the tested models, adjustments, and sensitivity analyses

Model	Adjustment	Sensitivity analysis
Adult BMI	Age, sex, and nonoccupational physical activity	Additional adjustment for BMI at age 20
BMI at age 20	Age, sex, and nonoccupational physical activity	Additional adjustment for adult BMI
BMI change in strata of adult BMI	Age, sex, and nonoccupational physical activity	
Height	Age, sex, nonoccupational physical activity, and weight	
Adult trouser/skirt size	Age, sex, nonoccupational physical activity, and adult BMI	
Nonoccupational physical activity	Age, sex, and adult BMI	
Occupational physical activity (used in sex-specific analyses for men only)	Age, sex, and adult BMI	
Early-life energy restriction during the Hunger Winter	Age, sex, and adult BMI	1) Additional adjustment for BMI at age 20; 2) age-stratified analysis
Early-life energy restriction during the war years	Age, sex, and adult BMI	1) Additional adjustment for BMI at age 20; 2) age-stratified analysis
Early-life energy restriction during the economic depression	Age, sex, and adult BMI	1) Additional adjustment for BMI at age 20; 2) age-stratified analysis

not significantly differ by extent of *IGFBP* methylation ($P = 0.98$ and 0.08, respectively).

Colorectal cancer risk by extent of *IGFBP* methylation

Adult BMI and early-life energy restriction were associated with colorectal cancers with several methylated *IGFBP* genes after multivariable corrections (Table 4). For adult BMI, comparison of the highest versus lowest sex-specific tertiles gave HRs (95% CIs) for colorectal cancers with 0, 1, 2, and 3 methylated genes of 1.39 (0.88–2.19), 1.11 (0.77–1.62), 1.67 (1.17–2.38), and 2.07 (1.29–3.33), respectively. Significant linear trends were observed in associations between adult BMI and colorectal cancers with 2 and 3 methylated genes ($P < 0.01$). Adult BMI modeled per 5-unit increase corroborated the observed associations. BMI at age 20, BMI change since age 20 modeled in strata of adult BMI, height, adult trouser/skirt size, and nonoccupational physical activity were not associated with colorectal cancer risk by *IGFBP* methylation status, although there was a borderline significant inverse trend across nonoccupational physical activity categories in relation to colorectal cancer with 3 methylated *IGFBP* genes (P trend = 0.06). Exposure to early-life energy restriction during the Hunger Winter versus nonexposure gave HRs (95% CIs) for colorectal cancers with 0, 1, 2, and 3 methylated *IGFBP* genes of 1.01 (0.67–1.53), 1.03 (0.74–1.44), 0.72 (0.52–0.99), and 0.50 (0.32–0.78), respectively. A similarly decreased HR for colorectal cancer with 3 methylated *IGFBP* genes was observed in those exposed to energy restriction during the war years versus those nonexposed (HR, 0.59; 95% CI, 0.39–0.89). Having had an unemployed father during the economic depression was not significantly associated with any of the endpoints, yet strong inverse HRs were observed for colorectal cancers with 2 and 3 methylated *IGFBP* genes (HR, 0.59; 95% CI, 0.35–1.02 and HR, 0.59; 95% CI, 0.30–1.19, respectively). Additional adjustment for adult BMI in models for BMI at age 20 and *vice versa* did not essentially change results, nor did additional adjustment for BMI at age 20 in models for early-life energy restriction (data not shown). Age-stratified analyses for early-life energy restriction did not reveal essential differences in associations between individuals exposed at young or later age in early life, although there was diminished power in these analyses (data not shown).

In sex-specific analyses, shown in the Supplementary Tables S2 and S3, adult BMI was significantly associated with an increased risk of colorectal cancer with several methylated *IGFBP* genes in men but not women, although HRs in women were nonsignificantly increased. Height was significantly associated with an increased risk of colorectal cancer with several methylated *IGFBP* genes in women, but not men. Other indicators of body size and (non)occupational physical activity were not associated with colorectal cancer risk by *IGFBP* methylation status,

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Table 2. Characteristics of subcohort members and colorectal cancer cases in the NLCS (1989–1993)

	Subcohort members		Colorectal cancer cases[a]	
	***N* (%)**	**Mean (SD)**	***N* (%)**	**Mean (SD)**
Overall	4,658 (100)		652 (100)	
Case characteristics				
IGFBP2 methylated			265 (40.6)	
IGFBP3 methylated			262 (40.2)	
IGFBP7 methylated			468 (71.8)	
IGFBP methylation index				
0 genes methylated			122 (18.7)	
1 gene methylated			192 (29.5)	
2 genes methylated			211 (32.4)	
3 genes methylated			127 (19.5)	
Baseline characteristics[b]				
Anthropometry				
Adult BMI, kg/m^2				
Men		25.0 (2.6)		25.5 (2.8)
Women		25.1 (3.5)		25.7 (3.6)
BMI at age 20, kg/m^2				
Men		21.8 (2.4)		22.0 (2.3)
Women		21.4 (2.8)		21.5 (2.5)
BMI change since age 20, kg/m^2				
Men		+3.3 (3.0)		+3.5 (3.0)
Women		+3.7 (3.7)		+4.1 (3.7)
Height, cm				
Men		176.4 (6.7)		176.8 (6.9)
Women		165.1 (6.2)		166.4 (6.4)
Adult trouser/skirt size				
<median, sex-specific	1,694 (40.5)		189 (33.0)	
≥median	2,486 (59.5)		384 (67.0)	
Physical activity				
Nonoccupational physical activity				
≤30 min/d	995 (22.5)		131 (21.3)	
30–90	2,270 (51.4)		318 (51.7)	
>90	1,150 (26.1)		166 (27.0)	
Early-life energy restriction				
Exposure to the Hunger Winter (1944–45)				
Non-Western area	2,255 (58.0)		335 (63.7)	
Western area	1,631 (42.0)		191 (36.3)	
Exposure to the war years (1940–44)				
Rural area	1,573 (48.4)		244 (51.4)	
Urban area	1,679 (51.6)		231 (48.6)	
Economic depression (1932–40)				
Employed	3,702 (88.3)		534 (90.7)	
Unemployed	492 (11.7)		55 (9.3)	

[a]Cases with successful *IGFBP2*, *IGFBP3*, and *IGFBP7* methylation analyses.

[b]Numbers are excluding missing values on adult BMI and nonoccupational physical activity.

although there was a significant association between BMI at age 20 measured per 5-unit increase and colorectal cancers with 2 (but not 3) methylated genes in men. Early-life energy restriction was significantly inversely associated with having several methylated *IGFBP* genes in colorectal cancer in men and women. Supplementary Table 4 shows results by *IGFBP2*, *IGFBP3*, and *IGFBP7* methylation status to aid future literature comparison. Overall, these analyses were difficult to interpret as significant associations were not specific to colorectal cancers

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Table 3. Molecular and clinical characteristics of colorectal cancer cases by extent of *IGFBP* methylation in the NLCS (1989–1993)

	***IGFBP* methylation index**			
	0 genes methylated	**1 gene methylated**	**2 genes methylated**	**3 genes methylated**
Molecular characteristics				
Tumor instability type, *N* (%)				
MSI (including CIMP)	6 (7.1)	9 (5.9)	24 (14.4)	26 (24.5)
MSS CIMP	2 (2.4)	19 (12.5)	37 (22.2)	41 (38.7)
MSS non-CIMP	76 (90.5)	124 (81.6)	106 (63.5)	39 (36.8)
P for χ^2 test		<0.001[a]		
Clinical characteristics				
Age at diagnosis (y), mean (SD)	67.8 (4.5)	67.9 (3.9)	67.9 (4.5)	68.1 (4.1)
P for Kruskal–Wallis test		0.98		
Tumor localization, *N* (%)				
Proximal colon	29 (24.4)	47 (25.1)	73 (35.3)	64 (52.0)
Distal colon	43 (36.1)	74 (39.6)	70 (33.8)	21 (17.1)
Rectosigmoid	15 (12.6)	25 (13.4)	18 (8.7)	12 (9.8)
Rectum	32 (26.9)	41 (21.9)	46 (22.2)	26 (21.1)
P for χ^2 test		<0.001[a]		
TNM stage, N (%)				
Stage 1	6 (5.3)	14 (8.0)	17 (8.9)	11 (9.2)
Stage 2	30 (26.6)	36 (20.5)	42 (22.0)	19 (16.0)
Stage 3	69 (61.1)	120 (68.2)	119 (62.3)	73 (61.3)
Stage 4	8 (7.1)	6 (3.4)	13 (6.8)	16 (13.5)
P for χ^2 test		0.08		

[a]Remained statistically significant after a Bonferroni correction for multiple testing.

with methylated *IGFBP* genes, but were also observed for colorectal cancers with unmethylated *IGFBP* genes.

Discussion

This is the first study suggesting that adult BMI, height (in women only), and early-life energy restriction predict preferentially for colorectal tumors characterized by *IGFBP*-methylated genes. This study is an example of a molecular pathologic epidemiologic study in which dietary or lifestyle risk factors are investigated in relation to molecular changes in tumors (37). To fully appreciate our findings, absolute values of BMI should be considered. Using WHO criteria as a reference (24), we report that 97.2% of the participants in the lowest tertile in this study fell within the normal adult weight range (BMI, 18.5–<25.0 kg/m^2), and 80.3% of the participants in the highest tertile fell within the overweight range (BMI, 25.0–<30.0 kg/m^2). The significantly increased HRs for adult BMI were, thus, based on a fairly small contrast in BMI. Although we must be careful extrapolating findings, stronger associations may be expected in populations with a higher obesity prevalence if these allow for a larger BMI contrast to be made. With respect to BMI at age 20, 92.3% of the study participants had a BMI <25.0 kg/m^2, and it may be that there was too little contrast between individuals to detect associations.

Our results should be interpreted with caution because validation of our findings is needed. To our knowledge, there are no other experimental or observational data on body size, physical activity, and early-life energy restriction in relation to *IGFBP* methylation in colorectal tumors. The advantage of measuring *IGFBP* methylation instead of IGFBP expression in colorectal tumors is that methylation likely represents enduring change. However, the actual amount of bioavailable IGFBPs in the tumor may also depend on the influx of IGFBPs from the circulatory system and on factors such as IGFBP proteases (3), growth hormone, and insulin (2), and may, thus, be better reflected by IGFBP expression levels. Therefore, the investigation of body size, physical activity, and energy restriction in relation to IGFBP expression levels in colorectal tumors may be informative in addition to validation of the present results. Studies focusing on tissue-specific expression levels could also explain inconsistent findings with respect to associations between IGFBP blood levels and colorectal cancer risk (2, 38, 39), as IGFBP blood levels may not necessarily correlate with tissue-specific levels.

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Table 4. Multivariable-adjusted HRs and 95% CIs for colorectal cancer by extent of *IGFBP* methylation in relation to body size, physical activity, and early-life energy restriction in the NLCS (1989–1993)

		0 *IGFBP* genes methylated		1 *IGFBP* gene methylated		2 *IGFBP* genes methylated		3 *IGFBP* genes methylated	
	PY	*N* cases	HR[a] (95% CI)	*N* cases	HR[a] (95% CI)	*N* cases	HR[a] (95% CI)	*N* cases	HR[a] (95% CI)
Body size									
Adult BMI, kg/m^2									
T1, sex-specific[b]	7,181	33	1 (Reference)	56	1 (Reference)	53	1 (Reference)	27	1 (Reference)
T2	7,059	34	1.04 (0.64–1.69)	61	1.10 (0.76–1.59)	64	1.22 (0.84–1.77)	37	1.39 (0.84–2.29)
T3	7,079	45	1.39 (0.88–2.19)	61	1.11 (0.77–1.62)	87	1.67 (1.17–2.38)	57	2.07 (1.29–3.33)
P trend			0.16		0.57		0.004		0.002
Adult BMI, per 5 kg/m^2	21,319	112	1.26 (0.93–1.71)	178	1.19 (0.93–1.53)	204	1.39 (1.11–1.74)	121	1.34 (1.07–1.67)
BMI at age 20, kg/m^2									
T1, sex-specific[c]	6,187	28	1 (Reference)	49	1 (Reference)	48	1 (Reference)	26	1 (Reference)
T2	5,991	32	1.19 (0.71–2.00)	55	1.17 (0.79–1.73)	63	1.37 (0.93–2.02)	38	1.53 (0.92–2.53)
T3	6,030	35	1.32 (0.80–2.18)	42	0.90 (0.59–1.37)	60	1.32 (0.90–1.95)	33	1.35 (0.80–2.27)
P trend			0.27		0.64		0.15		0.25
BMI at age 20, per 5 kg/m^2	18,208	95	1.16 (0.81–1.65)	146	1.07 (0.79–1.45)	171	1.23 (0.94–1.60)	97	1.27 (0.91–1.78)
BMI change, per kg/m^{2d}									
Stratum: adult BMI <25 kg/m^2	7,895	36	0.98 (0.81–1.18)	62	0.86 (0.74–1.01)	59	0.90 (0.80–1.02)	34	0.87 (0.72–1.05)
Stratum: adult BMI ≥25 kg/m^2	8,209	49	1.00 (0.99–1.14)	73	1.00 (0.91–1.10)	97	1.03 (0.95–1.12)	55	0.94 (0.85–1.05)
Height, cm									
T1, sex-specific[e]	7,756	38	1 (Reference)	55	1 (Reference)	60	1 (Reference)	38	1 (Reference)
T2	7,261	42	1.12 (0.71–1.77)	60	1.13 (0.77–1.64)	66	1.05 (0.73–1.51)	36	0.93 (0.58–1.48)
T3	6,301	32	0.91 (0.56–1.49)	63	1.29 (0.86–1.95)	78	1.23 (0.84–1.79)	47	1.25 (0.77–2.01)
P trend			0.73		0.22		0.28		0.36
Height, per 5 cm	21,319	112	0.94 (0.81–1.10)	178	1.02 (0.90–1.16)	204	1.05 (0.93–1.19)	121	1.11 (0.95–1.30)
Adult trouser/skirt size									
<Median, sex-specific	8,230	41	1 (Reference)	57	1 (Reference)	57	1 (Reference)	34	1 (Reference)
≥Median	11,964	63	0.91 (0.57–1.43)	112	1.25 (0.86–1.82)	131	1.22 (0.84–1.75)	78	1.26 (0.78–2.04)
Physical activity									
Nonoccupational physical activity, min/d									
≤30	4,747	22	1 (Reference)	33	1 (Reference)	42	1 (Reference)	34	1 (Reference)
>30–90	11,002	58	1.21 (0.74–1.99)	86	1.18 (0.79–1.78)	109	1.18 (0.82–1.70)	65	0.90 (0.59–1.38)
>90	5,569	32	1.26 (0.73–2.19)	59	1.55 (1.00–2.38)	53	1.05 (0.69–1.60)	22	0.59 (0.33–1.03)
P trend			0.42		0.04		0.87		0.06
Early-life energy restriction									
Hunger Winter (1944–45)									
Non-Western area	10,891	57	1 (Reference)	88	1 (Reference)	117	1 (Reference)	73	1 (Reference)
Western area	7,899	41	1.01 (0.67–1.53)	65	1.03 (0.74–1.44)	59	0.72 (0.52–0.99)	26	0.50 (0.32–0.78)
War years (1940–44)									
Rural area	7,572	37	1 (Reference)	68	1 (Reference)	80	1 (Reference)	59	1 (Reference)
Urban area	8,148	49	1.21 (0.78–1.89)	67	0.89 (0.63–1.27)	77	0.90 (0.65–1.25)	38	0.59 (0.39–0.89)
Economic depression (1932–40)									
Employed	17,900	99	1 (Reference)	147	1 (Reference)	182	1 (Reference)	106	1 (Reference)
Unemployed	2,367	10	0.72 (0.37–1.41)	21	1.04 (0.65–1.67)	15	0.59 (0.35–1.02)	9	0.59 (0.30–1.19)

Abbreviations: PY, person-years at risk; T, tertile.

[a]Adjusted for age and sex. In addition, all models, except models for nonoccupational physical activity, were adjusted for nonoccupational physical activity; models for adult trouser/skirt size, nonoccupational physical activity, and early-life energy restriction were adjusted for adult BMI; models for height were adjusted for adult weight.

[b]The range in sex-specific tertiles of adult BMI was 16.1–23.9, 23.9–25.9, and 25.8–39.7 kg/m^2 in men and 14.5–23.5, 23.4–26.2, and 26.1–41.6 kg/m^2 in women.

[c]The range in sex-specific tertiles of BMI at age 20 was 12.0–20.8, 20.7–22.6, and 22.6–31.9 kg/m^2 in men and 13.0–20.3, 20.2–22.5, and 22.4–46.9 kg/m^2 in women.

[d]Excluding individuals with a negative BMI change since age 20. The range in BMI change was 0–12.1 and 0–11.5 kg/m^2 in men and women, respectively, with an adult BMI <25 kg/m^2, and 0–19.1 and 0–22.7 kg/m^2 in men and women, respectively, with an adult BMI ≥25 kg/m^2.

[e]The range in sex-specific tertiles of height was 147–173, 174–179, and 180–200 cm in men and 140–163, 164–168, and 169–186 cm in women.

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It is relatively unclear how *IGFBP* methylation correlates with CIMP and MSI, and whether observed associations might be confounded by alterations underlying methylation in general. CIMP and MSI are important phenotypes in colorectal cancer associated with methylation of tumor suppressor- and DNA repair genes. Previously, *IGFBP7* methylation correlated positively with CIMP (40), as did *IGFBP3* methylation, especially with MSS CIMP tumors (15). *IGFBP3* methylation has also been used as a CIMP marker (41). We observed that the percentage of MSI and MSS CIMP tumors increased with increasing numbers of methylated *IGFBP* genes, but that still 36.8% of colorectal cancer cases with 3 methylated *IGFBP* genes had MSS non-CIMP tumors. It might, therefore, be argued that *IGFBP* methylation does not simply tag MSI and CIMP phenotypes. This may be reflected in subtle differences in associations with body size, physical activity, and early-life energy restriction. Using NLCS data, MSI in colorectal cancer has been associated with height (42). CIMP in colorectal cancer was associated with early-life energy restriction (33) and BMI at age 20, but not adult BMI (43). We discussed in these articles that findings of associations between BMI at age 20, early-life energy restriction, and epigenetic changes in tumors may be logical, considering that epigenetic changes generally occur early in colorectal tumorigenesis (14) and considering that obesity has been associated with a chronic state of low-grade inflammation (44, 45), which in turn has been associated with methylation (46). If, thus, viewing epigenetic changes as intermediary to BMI and colorectal cancer, it seems plausible that body size in early life but not later life is associated with epigenetic changes. Other case–control studies found associations between BMI and MSS tumors but not MSI tumors (47) and between BMI and MSI-stable and MSI-low tumors but not MSI-high tumors (48). Furthermore, associations have been reported between BMI and CIMP-low colon tumors, but not CIMP-high colon tumors (49). No associations were observed between BMI and CIMP-high or -low rectal tumors (50). Given that MSI and CIMP phenotypes correlate strongly and groups are likely to overlap (51), these findings suggest that adult BMI is not associated with MSI and CIMP, which are thought to reflect methylation on a broader scale. In the present study, adult BMI and early-life energy restriction were associated with *IGFBP* methylation in colorectal cancer when analyzing men and women together. Sex-specific analyses in addition revealed associations with height in women. Associations were repeatedly in the hypothesized direction. These apparently contradictory results might also indicate that *IGFBP* methylation does not simply tag MSI and CIMP phenotypes in colorectal cancer. We should, therefore, consider the possibility that a phenotype characterized by several methylated *IGFBP* genes shares underlying factors with MSI and CIMP that cause methylation, but that this phenotype does not share other factors that eventually determine MSI and CIMP.

In terms of the potential relevance of these results for public health, future research may focus on whether markers such as *IGFBP* methylation can be detected in colorectal polyps and is representative of a defect likely to occur in future lesions. If so, this would open up the exciting possibility of the use of these markers as a biomarker for identifying individuals who might benefit from weight gain prevention. The significance of such potential benefit should be investigated in carefully designed intervention studies. So far, methylation has been shown an early event in colorectal tumorigenesis, but the methylation status of multiple adenomas ($N = 78$) within the same patients ($N = 26$) in a study on CIMP using the methylation markers *p16*, *MINT2*, and *MINT31* correlated weakly (52). However, this study did not include sessile serrated adenomas, and it may be important to focus on this adenoma type because these lesions often develop through a pathway characterized by methylation (53), whereas methylation might be more random in other lesions.

Major strengths of this study include the prospective design and completeness of follow-up, making selection and information bias unlikely. A particular strength is also that we based the primer location for determining *IGFBP* methylation on deep-sequencing results for methylation in colon cell lines, ensuring that primers covered methylation "hot spots." A limitation may be that subsite-specific associations could not be studied because of limited power, even though this study is among the largest prospective studies assessing molecular characteristics in colorectal cancer. A limitation may also be the single baseline measurement obtained by self-reports. However, self-reports of body size have been shown to have good validity (54), and our measures of physical activity cover a considerable period in the lives of study participants, which may be important as colorectal cancer development is a process of decades.

To conclude, adult BMI, height (in women only), and early-life energy restriction were associated with the risk of having a colorectal tumor characterized by *IGFBP* methylation. The findings as described in this article might eventually facilitate more targeted approaches to prevent obesity-related colorectal cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: P.A. van den Brandt, M. van Engeland, M.P. Weijenberg

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.C.J.M. Simons, P.A. van den Brandt, M. van Engeland, M.P. Weijenberg

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.C.J.M. Simons, P.A. van den Brandt, M. van Engeland

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Body Size, Physical Activity, Early-Life Energy Restriction, and Associations with Methylated Insulin-like Growth Factor–Binding Protein Genes in Colorectal Cancer

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